10 μg/ml CLN; lane 8, 25 nM 4HNE plus 1 μg/ml CLN; lane 9, 25 nM 4HNE plus 0.1 μg/ml CLN.

Figure 5. CLN reduces 4HNE-mediated activation of p53. PC12 cells were pre-treated with CLN or LAH and exposed to 4HNE. Three hours after treatment, cell lysates were analyzed by Western blot analysis. (B) p53; (A) corresponding α-tubulin. 4HNE (25 nM), CLN (10 μg/ml), LAH (10 μg/ml).

Figures 6A-6G. (A) Normal morphology of SH-SY5Y control cells. Cells are mostly clumped, non-contact inhibited (right arrow) with a few elongated cells present. Their refractability indicates they are healthy and growing normally. (B) Cells treated with Beta-amyloid (10 μg/ml added on day 5) that show its toxicity. Note small round granulated cells with little refractability. (C) Differentiated SH-SY5Y cells following treatment with CLN (0.1 μg/ml added on day 5 for 30 minutes). Touching cells are flat, contact inhibited (not clumped), left arrow, and more isolated cells are elongated and neuronal in appearance, right arrow. (D) Cells protected from toxic (apoptotic effect) of Beta-amyloid by treating with CLN (Colostrinin 0.1 μg/ml added on day 5 for 30 minutes + Beta-amyloid 10 μg/ml added on day 5). Cells are flat (upper arrow) or elongated (lower arrow) showing typical morphology of differentiated cells (see Fig. 6C). (E) Inhibition of toxicity (apototic activity) of Beta-amyloid by CLN treatment (Colostrinin 3 μg/ml added on day 5 for 30 minutes + Beta-amyloid 10 μg/ml added on day 5). Note flattened (bottom arrow) and elongated (upper arrow) cells typical of SH-SY5Y differentiated cells. (F) Toxic (apoptotic) effect of retinoic acid (20 μM added on day 1) on SH-SY5Y cells. The observed toxicity resembles cytopathology induced by viruses. Cytoplasmic bridging caused by shrinking of cells once in contact with each other (upper right arrows), shrunken granular cells (lower right arrow) and small round cells (lower left arrows). (G) Inhibition of toxic effect of retinoic acid by treatment of SH-SY5Y with CLN (20 μM retinoic acid added on day 1 + 1 μg/ml Colostrinin added on day 5 for 30 minutes). Cells are well organized showing typical morphology of differentiated SH-SY5Y cells, elongation (lower arrow) and flattening (upper arrow).

Figure 7. Analysis of apoptosis by flow cytometry. (A) Induction of apoptosis by 4HNE (100 nM). UL, upper left; UR, upper right: necrotic cells;